THE INHIBITORY EFFECT OF COPROPORPHYRINS ON AMINO ACID UPTAKE INTO PROTEINS BY PORPHYRIC LIVER CELLS

Genevieve S. INCEFY and Attallah KAPPAS
The Rockefeller University, New York, N.Y. 10021, USA

Received 30 March 1972

1. Introduction

Steroids, drugs and certain chemicals stimulate the synthesis of porphyrins in avian liver cells by inducing the de novo formation of δ-aminolevulinate synthetase (ALAS), the rate-limiting enzyme in the porphyrin–heme biosynthetic pathway [1–3]. This chemically-induced hepatic porphyria has proved to be a useful experimental model for examining pathogenetic mechanisms in the natural disorders of porphyrin metabolism in man. The excess porphyrins synthesized by avian liver cells treated with porphyrinogenic chemicals accumulate in these cells in very high concentrations [1, 4]; similarly high concentrations of porphyrins accumulate in the liver in certain of the human porphyrias. The pharmacological effects which these porphyrins may have on the various metabolic processes of hepatic cells are not known. In the course of other studies, we observed that in liver cells treated with the potent porphyrinogenic agent allylisopropylacetamide (AIA), the incorporation of ^{14}C-leucine into proteins *in vitro* was significantly less than in untreated cultures. This effect was unexpected in light of the known increase in liver enzyme synthesis which takes place in experimental porphyria [5, 6]; moreover the inhibition of ^{14}C-leucine incorporation into protein was observed only during the latter part of the induction period when porphyrins are present in great excess in the treated hepatic cells. Such inhibition was also noted when exogenous δ-aminolevulinic acid (ALA) was added to the cultures and transformed by the cell into porphyrins.

These findings led us to examine the direct effects of porphyrins on amino acid incorporation into protein by cultured liver cells. The results of these experiments, which indicate that excessive concentrations of coproporphyrins in liver cells inhibit amino acid uptake into proteins by an as yet unknown mechanism, are reported here.

2. Experimental

Chick embryo liver cells were grown in primary cultures on 16 mm cover slips or in 100 mm diam. plastic petri dishes by the method of Granick [1]. The medium was replaced after 15–24 hr at which time various compounds were added to the cultures and incubation continued for an additional 18–24 hr. The culture medium was removed immediately prior to labelling with ^{14}C-leucine, frozen and saved for quantitative analysis of porphyrins utilizing our previously described procedure [7]. Warm (37°) leucine-free medium (Microbiological Associates) and ^{14}C-L-leucine (New England Nuclear, 260 mCi/mmole) were then immediately added to the cultures and the cells exposed to ^{14}C-leucine (450,000 cpm/ml) for 30 min. After this pulse-labelling, the cells were rinsed with appropriate buffer and processed as described under tables 1 and 2. Radioactivity was determined in a mixture of Omnifluor (New England Nuclear) and toluene with an efficiency of approx. 98% in a Packard scintillation spectrometer. The quantitative values reported are based on the means of ^{14}C-leucine incorporation observed in 5 vials and are representative of replicate experiments. Protein was determined by the method of Lowry et al. [8] with bovine serum albumin as standard.

Table 1
Effect of porphyrinogenic compounds and coproporphyrins on the *in vitro* incorporation of ^{14}C-L-leucine into proteins of chick embryo liver cell cultures.

Exp.	Cells	Incubation time	Porphyrins		^{14}C-L-leucine incorporation			
		(hr)	(pmoles/ml	± S.D.)	(cpm/vial	± S.D.)	Inhibition (%)	*P*
1	Control	24	17.1	± 6.8	4987	± 684	0	
	AIA	24	90.3	± 5.1	3217	± 508	−36	<0.005
2	Control	22	12.9	± 7.1	6990	± 653	0	
	AIA	22	46.9	± 7.9	3658	± 420	−48	<0.001
	ALA	22	97.3	± 16.8	2588	± 516	−63	<0.001
3	Control	18	10.8	± 2.2	5488	± 347	0	
	Copro III	18	260.	± 4.1	3614	± 400	−34	<0.005
4	Control	24	17.7	± 1.2	6705	± 1187	0	
	Copro I	24	152.	± 1.2	3517	± 763	−48	<0.001

Liver cells were grown on cover slips for 22 hr prior to addition of 30 μg of AIA or 100 μg of ALA in 5 λ of H_2O/ml of medium or 150–260 pmoles of copro I and III, respectively, per ml medium with subsequent incubation for the times indicated. Cells were labelled for 30 min with ^{14}C-leucine as described in the text, washed several times in cold phosphate-saline, fixed 10 min in acetic acid–alcohol at 4°, extracted 1 hr with 2% HClO and rinsed extensively with water according to the procedure of Franklin [10]. Cells were solubilized in 0.5 ml of soluene (Packard) and radioactivity determined as described in the text. The porphyrin content of the medium is the mean of 5 individually assayed culture vials with copro III as standard [7]; ^{14}C-leucine incorporation by liver cells is the mean of 5 cover slips; S.D. is the standard deviation. *P* values of *t*-test are highly significant.

3. Results and discussion

The results in table 1 show that addition of the porphyrinogenic chemical AIA to avian embryo hepatocytes grown in primary cultures stimulated the synthesis of porphyrins, as expected, their concentration in the medium reaching 5 times the levels in control cultures after 24 hr of incubation. The concentration of porphyrins in the cells themselves exceeds that in the medium [1, 4]. However ^{14}C-leucine incorporation into protein by these AIA-induced liver cells was decreased significantly as compared with controls (exp. 1, 2). The inhibition was observed consistently in similar experiments, but occurred only towards the latter part of the incubation period when porphyrins are known to accumulate in the cells and medium of the cultures [1, 4]. To ascertain that this inhibition was not due to some artifact specifically involving ^{14}C-leucine incorporation *in vitro*, in other experiments cells induced with AIA were pulse-labelled with a ^{14}C-amino acid mixture or with ^{14}C-guanido-L-arginine, an amino acid which is not reutilized [9], and similar results were obtained. The results with the latter amino acid exclude artifacts resulting from re-utilization of the label and also suggest that the inhibition effect relates to protein biosynthetic rather than catabolic or other processes in the cell.

To examine further the relationship between the presence of excess porphyrins in the AIA-treated cultures and the incorporation of ^{14}C-leucine by the cell monolayer, synthesis of porphyrins was increased in control cultures by exogenous addition of ALA, the product of the enzymatic reaction catalyzed by ALAS. No inhibitory effect on ^{14}C-leucine incorporation *in vitro* was observed when ALA was present in the cultures for 1 hr; however when the ALA was allowed to be transformed into porphyrins by the cells during a further 22 hr of incubation, a pronounced inhibition of ^{14}C-leucine incorporation into protein was observed. This inhibition was of a greater degree than that observed in the AIA-treated cultures (table 1, exp. 2). If porphyrins in the liver cell cultures produced this inhibition of protein synthesis, they would be expected to act directly in control cultures, as well as after having been formed by the cells from ALA or via induction of ALAS by AIA. The results shown in table 1

Table 2
Inhibition of ^{14}C-L-leucine incorporation into protein from homogenates of chick embryo liver cell cultures treated with AIA and coproporphyrin III.

Cells	Incubation time	Porphyrins		Protein		^{14}C-L-leucine incorporation				Inhibition	
	(hr)	(pmole/ml	± S.D.)	(μg/ml	± S.D.)	(cpm/ml	± S.D.)	(cpm/μg	± S.D.)	(%)	*P*
Control	24	4.03	± 0.3	528	± 1.8	19708	± 780	37.3	± 1.5	0	
AIA	24	59.5	± 0.9	480	± 1.8	12769	± 280	25.6	± 0.6	−29	<0.001
Copro III	24	94.1	± 1.2	484	± 3.5	14215	± 93	29.4	± 0.2	−21	<0.005

Liver cells were grown in petri dishes (100 mm in diam.) for 15 hr prior to addition of AIA and copro III (50 μg and 177 pmole/ml of medium, respectively) with subsequent incubation for 24 hr. Medium was removed at the end of the incubation and its coproporphyrin content determined [7]. The cell monolayer was then labelled with ^{14}C-leucine for 30 min as described in the text. Cells were rinsed twice with 0.05 M Tris, pH 7.4, scraped from the bottom of plates and homogenized in this buffer. Triplicate aliquots of each homogenate fraction were precipitated with 10% TCA, washed 3 times with 5% TCA by centrifugation and solubilized in 0.5 ml of soluene. Radioactivity was determined as described in the text. S.D. is the standard deviation; *P* values of the *t* test are highly significant.

(exp. 3, 4) show this to be the case. In these experiments, and similar ones not reported, coproporphyrins I and III (copro I and III) consistently and significantly inhibited ^{14}C-leucine incoporation into protein; hemin, at comparable concentration did not have this effect.

Inhibition with copro I and III was apparent as early as 1 hr after they were added to the cultures, and the effect persisted as long as the liver cells were exposed to the compounds. Since determination of the protein content of the ^{14}C-labelled liver cells grown on coverslips was not feasible because of the need for fixation and isotope counting of the monolayer the effect of AIA and copro III on ^{14}C-leucine incorporation was also determined in homogenates of cells grown in 100 mm diameter plastic petri dishes. The results expressed as "specific activity" (with respect to the protein content of the labelled aliquots of homogenized cells) are shown in table 2; inhibition of ^{14}C-leucine uptake is again apparent in the porphyrin treated cell cultures verifying the observations made in the experiments shown in table 1.

The mechanism for the coproporphyrin effect on the uptake of amino acids into proteins by liver cells is not yet established. Although porphyrins have been reported to have an affinity for plasma proteins and lipoproteins [11, 12] they are also chelating agents for various divalent ions [13]. The culture medium used in these experiments however contains a large excess of Mg^{2+}, a cation essential for activation of various enzymes and for protein biosynthesis. A porphyrin interaction with plasma membranes to prevent entry of labelled amino acids into the liver cell seems unlikely since ^{14}C-leucine incorporation by cytosol fractions of embryonic avian hepatocytes was also inhibited by these compounds.

Whatever their proximate mode of action, the ability of coproporphyrins to inhibit the rate of amino acid incorporation into liver cell proteins is significant and needs to be taken into account when evaluating kinetic parameters of specific proteins, i.e. enzymes, in porphyric avian hepatocytes.

Acknowledgements

We wish to thank Mrs. Maureen Morgan and Miss Mary Horan for skilled technical assistance and Miss Ann Quatela for typing the manuscript. The work was supported by NIH Grant No. HD 04313 and ES00621.

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